

Appendix B

PENDING CLAIMS

What is claimed is:

1. A recombinant cell comprising:
 - (i) a receptor that, upon ligand stimulation, activates an endogenous signal transduction pathway; and
 - (ii) a heterologous DNA construct comprising a gene encoding a protein that activates the signal transduction pathway, which gene is operatively linked to a promoter that is responsive to activation of the signal transduction pathway;wherein stimulation of the receptor by a ligand leads to expression of the heterologous DNA construct encoding the protein that activates the signal transduction pathway such that signals generated by ligand binding to the receptor are amplified.
2. The cell of claim 1, which is a yeast cell.
3. The cell of claim 1, which is a mammalian cell.
4. The cell of claim 1, wherein the receptor is a G protein coupled receptor.
5. The cell of claim 4, wherein the G protein coupled receptor is a heterologous G protein coupled receptor.
6. The cell of claim 1, wherein the endogenous signal transduction pathway is a mitogen activated protein kinase (MAPK) pathway.

7. The cell of claim 2, wherein the endogenous signal transduction pathway is a yeast pheromone response pathway.

8. A recombinant yeast cell comprising :

- (i) a heterologous G protein coupled receptor that, upon ligand stimulation, activates an endogenous yeast pheromone response pathway; and
- (ii) a heterologous DNA construct comprising a gene encoding a protein that activates the yeast pheromone response pathway, which gene is operatively linked to a promoter that is responsive to activation of the yeast pheromone response pathway

wherein stimulation of the receptor by a ligand leads to expression of the heterologous DNA construct encoding the protein that activates the yeast pheromone response pathway such that signals generated by ligand binding to the receptor are amplified.

9. The yeast cell of claim 8, wherein the gene encoding a protein that activates the yeast pheromone response pathway is STE5.

10. The yeast cell of claim 8, wherein the gene encoding a protein that activates the yeast pheromone response pathway is STE4.

11. The yeast cell of claim 8, wherein the gene encoding a protein that activates the yeast pheromone response pathway is STE12.

12. The yeast cell of claim 8, wherein the gene encoding a protein that activates the yeast pheromone response pathway is STE11.

13. The yeast cell of claim 8, wherein the gene encoding a protein that activates the yeast pheromone response pathway is a dominant truncation allele of STE20.

14. The yeast cell of claim 8, wherein the gene encodes a hypersensitive mutant form of the protein that activates the yeast pheromone response pathway.
15. The yeast cell of claim 14, wherein the hypersensitive mutant form is a mutant Ste5 protein.
16. The yeast cell of claim 15, wherein the mutant Ste5 protein has a T52M mutation or T52M,S18R mutations.
17. The yeast cell of claim 14, wherein the hypersensitive mutant form is a mutant Ste4 protein.
18. The yeast cell of claim 17, wherein the mutant Ste4 protein has a mutation selected from the group consisting of G124D; W136G; W136R; Δ L138; W136R,L138F; and W136G,S151C.
19. The yeast cell of claim 14, wherein the hypersensitive mutant form is a mutant Ste11 protein.
20. The yeast cell of claim 19, wherein the mutant Ste11 protein has a T596I mutation (allele Ste11-4) or a P278S mutation (allele Ste11-1).
21. The yeast cell of claim 14, wherein the hypersensitive mutant form is a mutant Fus3 protein.
22. The yeast cell of claim 21, wherein the mutant Fus3 protein has a I161L mutation.
23. The yeast cell of claim 8, wherein the promoter that is responsive to activation of the yeast pheromone response pathway is selected from the group consisting of: FUS1, AGA1, FAR1, and FUS2.
24. The yeast cell of claim 23, wherein the promoter is a FUS1 promoter.

25. The yeast cell of claim 8, wherein an endogenous yeast gene encoding a protein that negatively regulates the yeast pheromone system pathway is mutated to render the protein nonfunctional.
26. The yeast cell of claim 25, wherein the endogenous gene that is mutated encodes a phosphatase that negatively regulates the yeast pheromone system pathway.
27. The yeast cell of claim 26, wherein the endogenous gene encoding the phosphatase is selected from the group consisting of: MSG5, PTP2, and PTP3.
28. The yeast cell of claim 8, which further comprises a reporter gene construct that produces a detectable signal upon activation of the yeast pheromone response pathway.
29. The yeast cell of claim 8, wherein the heterologous DNA construct is carried by a high copy number plasmid.
30. The yeast cell of claim 8, wherein the heterologous DNA construct is carried by a low copy number plasmid.
31. The yeast cell of claim 8, which is a *Saccharomyces cerevisiae* cell.
32. A recombinant yeast cell comprising :
 - (i) a heterologous G protein coupled receptor that, upon ligand stimulation, activates an endogenous yeast pheromone response pathway; and
 - (ii) a heterologous DNA construct comprising a STE5 or STE4 gene encoding a Ste5 or Ste4 protein, respectively, that activates the yeast pheromone response pathway, which gene is operatively linked to a FUS1 promoter that is

responsive to activation of the yeast pheromone response pathway wherein stimulation of the receptor by a ligand leads to expression of the heterologous DNA construct encoding the Ste5 or Ste4 protein that activates the yeast pheromone response pathway such that signals generated by ligand binding to the receptor are amplified.

33. The yeast cell of claim 32, wherein the gene encodes a hypersensitive mutant form of the Ste5 or Ste4 protein that activates the yeast pheromone response pathway.

34. The yeast cell of claim 32, wherein an endogenous yeast gene encoding a protein that negatively regulates the yeast pheromone system pathway is mutated to render the protein nonfunctional.

35. The yeast cell of claim 34, wherein the endogenous gene that is mutated encodes a phosphatase that negatively regulates the yeast pheromone system pathway.

36. The yeast cell of claim 35, wherein the endogenous gene encoding the phosphatase is selected from the group consisting of: MSG5, PTP2, and PTP3.

37. The yeast cell of claim 32, which further comprises a reporter gene construct that produces a detectable signal upon activation of the yeast pheromone response pathway.

38. The yeast cell of claim 32, wherein the heterologous DNA construct is carried by a high copy number plasmid.

39. The yeast cell of claim 32, wherein the heterologous DNA construct is carried by a low copy number plasmid.

40. The yeast cell of claim 32, which is a *Saccharomyces cerevisiae* cell.

41. A recombinant yeast cell comprising:
a heterologous G protein coupled receptor that, upon ligand stimulation,
activates an endogenous yeast pheromone response pathway,
wherein an endogenous yeast gene encodes a phosphatase that negatively regulates the yeast
pheromone system pathway is mutated to render the protein nonfunctional such that signals
generated by ligand binding to the receptor are amplified.
43. The yeast cell of claim 41, wherein the endogenous gene encoding the phosphatase is
selected from the group consisting of: MSG5, PTP2, and PTP3.
44. A recombinant yeast cell comprising :
a heterologous G protein coupled receptor that, upon ligand stimulation,
activates an endogenous yeast pheromone response pathway,
wherein an endogenous FUS3 gene is mutated to encode a supersensitive form of Fus3 protein
such that signals generated by ligand binding to the receptor are amplified.
45. The yeast cell of claim 44, wherein the supersensitive form of Fus3 protein has a I161L
mutation.
46. An assay to identify compounds that modulate the activity of a receptor, comprising:
(i) providing a recombinant cell as claimed in claim 1, 8, 32, or 41, wherein a detectable
signal is produced in the cell upon stimulation of the receptor;
(ii) contacting the cell with a test compound; and
(iii) identifying a compound which induces a change in the detectable signal in the cell,
such a change indicating that the compound modulates the activity of the receptor.
47. The assay of claim 46, wherein the cell comprises a reporter gene construct that produces
a detectable signal upon receptor stimulation.

48. The yeast cell of claim 8, wherein the G protein coupled receptor is STE2.
49. The yeast cell of claim 28, wherein the reporter gene is lacZ.
50. The yeast cell of claim 28, wherein the reporter gene construct that produces a detectable signal is a FUS-1 promoter operatively linked to lacZ.

51. (New) A recombinant yeast cell comprising:

(i) a heterologous G-protein coupled receptor that, upon ligand stimulation, activates the endogenous yeast pheromone response pathway; and

(ii) a heterologous DNA construct comprising a gene encoding STE5, which STE5 activates the yeast pheromone response pathway, which gene is operably linked to a promoter that is responsive to activation of the yeast pheromone response pathway, wherein stimulation of the receptor by a ligand leads to expression of the gene encoding STE5 that activates the yeast pheromone response pathway such that signals generated by ligand binding to the receptor are amplified.

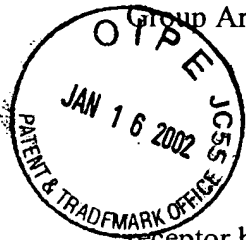
52. (New) A recombinant yeast cell comprising:

(i) a heterologous G-protein coupled receptor that, upon ligand stimulation, activates the endogenous yeast pheromone response pathway; and

(ii) a heterologous DNA construct comprising a gene encoding a protein that activates the yeast pheromone response pathway, which gene is operably linked to a promoter that is responsive to activation of the yeast pheromone response pathway, wherein stimulation of the

Serial No.: 09/378,046

Group Art No.: 1646



- 23 -

receptor by a ligand leads to expression of the gene encoding the protein that activates the yeast pheromone response pathway such that signals generated by ligand binding to the receptor are amplified, wherein said gene is selected from the group consisting of STE4, STE5, STE11, STE12, STE20 and FUS3.